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Bacterial 'histone-like protein I' (HLP-I) is an outer membrane constituent?

Laura Hirvas, Jack Coleman*, Pertti Koski and Martti Vaara

Department of Bacteriology and Immunology, University of Helsinki, 00290 Helsinki, Finland and **Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center, New Orleans, LA 70112, USA*

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The nucleoid-associated 'histone-like protein I' (HLP-I) protein of *E. coli* was found to be homologous with the cationic 16-kDa outer membrane protein OmpH of *Salmonella typhimurium*. Deduced from the nucleotide sequence, the HLP-I protein has 91% identical residues with the OmpH protein. Both proteins have very similar cleavable signal sequences. The nucleotide sequence similarity between the corresponding genes *hlpA* and *ompH* is 87%. The *ompH* gene is located in a gene cluster resembling the *hlpA*-ORF$_{17}$ region of *E. coli* which is close to the *Ipx* genes involved in the biosynthesis of lipopolysaccharides. The localization of the OmpH/HLP-I protein in the cell is discussed.

ompH gene; *hlpA* gene; Outer membrane protein; DNA binding protein; Bacterial chromatid; *(Escherichia coli; Salmonella typhimurium)*

1. INTRODUCTION

Many proteins are found associated with 'chromatin' isolated directly from bacterial lysates [1–3]. Two of them, the basic 6 kDa HU protein and the neutral 15 kDa H-NS protein (probably identical to another chromatin-associated protein, H1) are possibly integral constituents of the bacterial nucleoid whereas many others might be artificially bound to DNA during the isolation process [3].

A 17-kDa cationic nucleoid-associated *E. coli* protein has recently been isolated, and its gene cloned and sequenced, by Holck and Kleppe [4]. It is apparently identical to the histone-like protein HLP-I, studied a decade ago by Lathe et al. [5,6]. Accordingly, the gene designation *hlpA* has been adapted for the cloned structural gene and the product is being called as the HLP-I protein. Even though the HLP-I protein has been regarded a constituent of bacterial nucleoid [1,4], the evidence is still incomplete. Especially unexpected was the finding that the protein exists as a precursor which has a signal sequence [4]. This is characteristic of precursor proteins which are secreted (e.g., in *Enterobacteriaceae*, the periplasmic proteins and the outer membrane proteins). Thus far, no natural proteins have been mentioned which have a signal sequence-carrying precursor form but constantly remain cytoplasmic [7,8]. Furthermore, both the isolated HLP-I and approx. 95% of HLP-I synthesized by the high-copy *hlpA*-carrying plasmid (pGAH317) lack the signal sequence [4]. This indicates that the HLP-I preprotein is cleaved by the signal peptidase, a membrane protein which is part of the export machinery for secreted proteins [7,8]. However, when isolated membranes of *E. coli* which carries pGAH317 were analyzed by immunoblotting for the presence of HLP-I, only trace amounts of the protein were found [4].

In this paper, we show that the HLP-I protein is the *E. coli* homologue of the cationic 16-kDa protein which we have isolated from the outer membrane of *S. typhimurium* [9,10]. Furthermore, we will show that also the corresponding genes (*hlpA*, *ompH*) are very homologous and that *hlpA* is located in a larger gene cluster homologous to that where *ompH* lies. These data might suggest that HLP-I is an outer membrane protein.

Correspondence address: M. Vaara, Department of Bacteriology and Immunology, University of Helsinki, Haartmaninkatu 3, 00290 Helsinki, Finland

Abbreviations: aa, amino acid(s); LPS, lipopolysaccharide; nt, nucleotide(s); OM, outer membrane; ORF, open reading frame

2. EXPERIMENTAL

2.1. *DNA techniques*

The nt sequences were determined with the dideoxy chain-termination method of Sanger et al. [11]. The strands were sequenced directly as in [10] using denatured pUC plasmids as templates [12]. Both the pUC universal primers and specific synthetic oligodeoxyribonucleotides were used as primers.

A DNA probe was prepared from the *PstI-EcoRV* fragment of *S. typhimurium ompH* in pUCHS14 (537 nt, containing most of the structural gene) by nick translation [13]. The chromosomal DNA of *E. coli* K-12 strain JM109 [14] was isolated as in [9]. In Southern hybridization [9], the hybridization was done at 37°C overnight in 5 × SSC, 0.5% SDS, 5 × Denhardt's solution, 60% formamide. The

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                1         10         20         30         40         50         60
ECOHLPI.AMI     MKKWLLAAGLGLALATSAQAADKIAIVNMGSLFQQVAQKTGVSNTLENEFKGRASELQRM
                :::::::::::::. ::::::::::::::: :::::::::::::::::::::::::::.:.:
STMOMPH.AMI     MKKWLLAAGLGLAMVTSAQAADKIAIVNMGNLFQQVAQKTGVSNTLENEFKGRAAELQKM
                1         10         20         30         40         50         60

               61         70         80         90        100        110        120
ECOHLPI.AMI     ETDLQAKMKKLQSMKAGSDRTKLEKDVMAQRQTFAQKAQAFEQDRARRSNEERGKLVTRI
                ::::::.:: .:::::::::::::::::::::::::::: :::::::::: ::::::
STMOMPH.AMI     ETDLQSKMQRLQSMKAGSDRTKLEKDVMSQRQTFAQKAQAFEKDRARRSNEERNKLVTRI
               61         70         80         90        100        110        120

              121        130        140        150        160
ECOHLPI.AMI     QTAVKSVANSQDIDLVVDANAVAYNSSDVKDITADVLKQVK
                ::::: :::.:.:::::::::::::::::::::::::::::
STMOMPH.AMI     QTAVKKVANDQSIDLVVDANTVAYNSSDVKDITADVLKQVK
              121        130        140        150        160
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Fig.1. Alignment of the HLP-I protein of *E. coli* (ECOHLPI) and the OmpH protein of *S. typhimurium* (STMOMPH). Besides identical amino acids (marked with a double dot), also 'equivalent' amino acids (grouped as A, G, S, T; E, D, N, Q; R, K; M, L, I, V; F, Y, W; marked with a single dot) are shown.

hybridized filter was washed at 37°C in 5 × SSC, 0.5% SDS for 1 h and in 2 × SSC, 0.5% SDS for 30 min. All other DNA techniques were as described in [9,10].

2.2. *Computer analyses*

Analysis of the nucleotide sequence data and amino acid sequence data were performed using the HIBIO DNASIS and PROSIS computer program packages (Pharmacia, Bromma, Sweden). The hydrophobicity profile was calculated according to Kyte and Doolittle [15] with the window width of 5 amino acids. The secondary structure was predicted according to Chou, Fasman and Rose with the PROSIS program.

3. RESULTS

3.1. *HLP-I and OmpH proteins are highly homologous*

Fig.1 compares the deduced amino acid sequences of the OmpH protein of *S. typhimurium* [9,10] and the HLP-I protein of *E. coli* [4]. Both polypeptides are 161 aa long. The homology between the peptides is 91%. Both peptides have almost identical 20 aa long N-terminal sequences typical of signal peptides (i.e. a short basic part, Met-Lys-Lys, followed by a long hydrophobic stretch). The mature OmpH [9,10] and HLP-I [1,4,16] proteins lack the signal part; amino acid sequencing of both the isolated OmpH [9] and HLP-I [1,16] proteins reveal the following N-terminal sequence: ADKIAIVNMG.

The hydrophobicity profile of the OmpH protein was found to be very similar to that of the HLP-I protein (data not shown). Furthermore, both proteins had almost identical charge distribution and predicted secondary structure (data not shown).

3.2. *The* ompH *gene of* S. typhimurium *is located in a gene cluster resembling the* E. coli hlpA *ORF$_{17}$ region*

The gene which encodes the *E. coli* HLP-I protein has been called as *skp* or *firA* [4,6]. The current gene

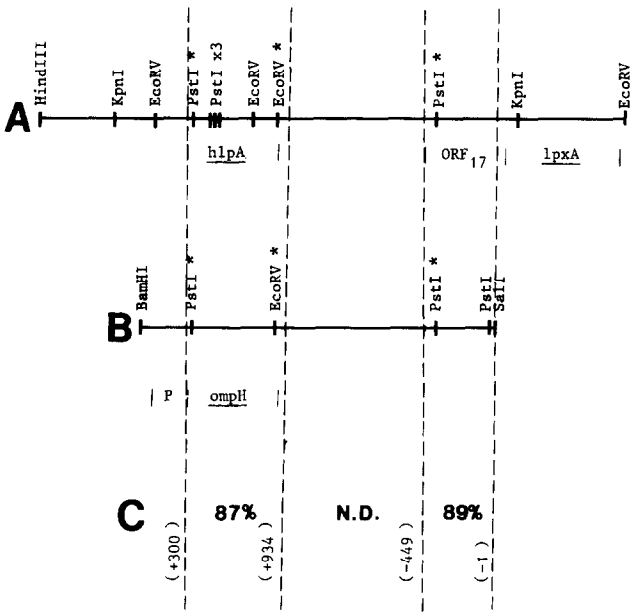

Fig.2. (A,B) Gene organization in the *hlpA*-ORF$_{17}$-*lpxA* region of *E. coli* (A; [6,16]) and the corresponding region of *S. typhimurium* (B; [9,10] and this study). The conserved restriction sites are indicated with an asterisk (*). (C) Nucleotide identity between the gene areas shown in panels A and B. (The numbers in parentheses marked with + or − refer to nt positions in the *S. typhimurium* gene area, read from the 5′ end of the informative and noninformative strand, respectively.) N.D., not determined.

designation is proposed to be *hlpA* according to the upcoming edition of the *E. coli* genetic map by B. Bachmann. Fig.2 shows the restriction maps and gene organizations of the *hlpA/ompH* region, as previously shown separately for *E. coli* [6,16] and *S. typhimurium* [10]. Within the *ompH/hlpA* gene area shown in fig.2, the nucleotide identity is 87% (82 nt from the total of 635 are different).

Both genes have an identical Shine-Dalgarno sequence (AGGAGTT), an open reading frame of 483 nt starting by the alternative start codon GTG, and, after the structural gene, an identical 26 nt long region of hyphenated dyad symmetry (see the original nucleotide sequence publications [4,9,10]).

We also used a nick-translated DNA-probe prepared from the *S. typhimurium ompH* (see section 2) to detect in Southern blotting homologous gene fragments from the *E. coli* K-12 (strain JM109) genome, digested with various restriction enzymes. The probe detected a 1.4 kb *Pst*I-*Pst*I fragment. We cloned this fragment. Its 5′ terminal sequence (340 bp sequenced in both directions) was 100.0% identical to the published sequence [4] of the *E. coli hlpA* gene (data not shown). Thus, that *E. coli* sequence has now been verified.

Both bacteria contain an approx. 1050 bp *Eco*RV-*Pst*I fragment just downstream of the *hlpA/ompH* gene (fig.2). The 3′ terminus of the *E. coli* fragment and its flanking downstream region are known to contain the codons for an open reading frame encoding a putative 17.3 kDa protein (ORF_{17}) [6,16]. In fig.2, we have compared the sequence of the *E. coli* ORF_{17} region with the corresponding *S. typhimurium* region (sequenced for this study). Within the 449 bp long region, the sequence similarity was 89%. Thus far, the nt sequence of the region between *E. coli hlpA* and ORF_{17} has not been published, so comparisons are not made regarding to that gene area.

4. DISCUSSION

In this communication, we showed that the histone-like cationic 17 kDa protein HLP-I of *E. coli* is the homologue of the cationic 16-kDa protein OmpH of the outer membrane of *S. typhimurium*. This was a surprising finding because HLP-I is believed to be a constituent of bacterial chromatin [1,4]. We have earlier shown that the outer membrane of *E. coli* contains a protein apparently very similar to the OmpH protein of *S. typhimurium* [9]. Taken together, this suggests that the *E. coli* outer membranes isolated by us contain HLP-I.

The LPS component of the OM is a notably anionic compound with several free phosphate and carboxyl groups per molecule. Also DNA is polyanionic. Due to its cationicity, the OmpH/HLP-I protein could, in bacterial lysates, be artificially bound to LPS, or DNA, or both. However, the presence of a cleavable signal sequence in the preprotein strongly suggests that the most likely localization of the mature protein in intact cells would be the cell envelope. As reviewed earlier by us [9], the OmpH protein is probably also homologous to the LPS-binding protein of Geyer et al., which is believed to be an outer membrane protein [17]. Empirical evidence suggests that the LPS-binding protein is antigenically exposed on the outer surface of the OM [17]. Furthermore, the *ompH/hlpA* gene lies very close (distance below 2.0 kb) to the *Ipx* genes involved in the biosynthesis of the lipid A component of LPS [6,16]. Whether the genes are functionally coupled, should be studied. The mechanism of LPS translocation from the cytoplasmic membrane to the outer leaflet of the OM has not yet been elucidated. It would be interesting to study whether the OmpH/HLP-I protein is involved in this stage of LPS biosynthesis.

Could the OmpH/HLP-I protein be simultaneously an outer membrane protein and a DNA-binding nucleoid protein? Even this possibility cannot be ruled out yet because, in several detailed studies, cell fractionation has produced cell envelope – DNA complexes (for reviews, see [3,18]). The isolated OM has been shown to contain DNA fragments of the replicative origin (*oriC*) of the chromosome [19,20].

Accordingly, no conclusive data can be given at this stage regarding to the cellular localization of the OmpH/HLP-I protein. It is clear that a critical study of conditionally lethal mutants which produce defective OmpH/HLP-I will remarkably help in settling the question.

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